(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 March 2003 (20.03.2003)

PCT

(10) International Publication Number
WO 03/022324 A1

(51) International Patent Classification[7]: A61L 31/16, 27/54

(21) International Application Number: PCT/US02/28798

(22) International Filing Date:
10 September 2002 (10.09.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/950,307 10 September 2001 (10.09.2001) US

(71) Applicant: ABBOTT LABORATORIES [US/US]; D-377 AP6A-1, 100 Abbott Park Road, Abbott Park, IL 60064-6008 (US).

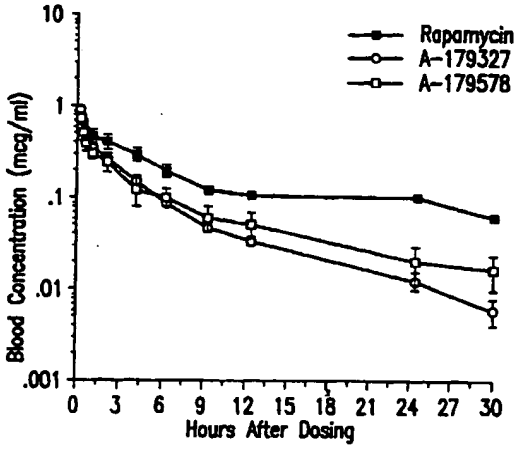
(72) Inventors: MOLLISON, Karl, W.; 4020 N Harvard Ave, Arlington Heights, IL 60004 (US). LECAPTAIN, Angela, M.; 555 E Bridlewood Ln, Oak Creek, WI 53154 (US). BURKE, Sandra, E.; 1025 Regency Lane, Libertyville, IL 60048 (US). CROMACK, Keith, R.; 18066 W Banbury Dr, Gurnee, IL 60031 (US). TARCHA, Peter, J.; 21651 Gelden Road, Lake Villa, IL 60046 (US).

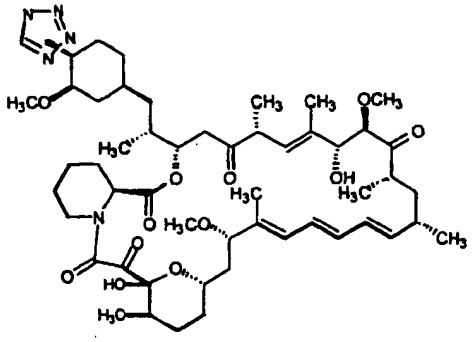
(74) Agents: WEINSTEIN, David, L. et al.; D-377 AP6A-1, 100 Abbott Park Road, Abbott Park, IL 60064-6008 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

[Continued on next page]

(54) Title: MEDICAL DEVICES CONTAINING RAPAMYCIN ANALOGS





(I)

(57) Abstract: A medical device comprising a supporting structure having a coating on the surface thereof, the coating containing a therapeutic substance, such as, for example, a drug. Supporting structures for the medical devices that are suitable for use in this invention include, but are not limited to, coronary stents, peripheral stents, catheters, arterio-venous grafts, by-pass grafts, and drug delivery balloons used in the vasculature. Drugs that are suitable for use in this invention include, but are not limited to,

WO 03/022324 A1

(84) **Designated States** *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

MEDICAL DEVICES CONTAINING RAPAMYCIN ANALOGS

This application is a continuation-in-part of United States Serial No. 09/433,001, filed November 2, 1999, which is a divisional of U. S. Serial No. 09/159,945, filed September 24, 1998, now United States Patent No. 6,015,815, incorporated herein by reference.

Technical Field

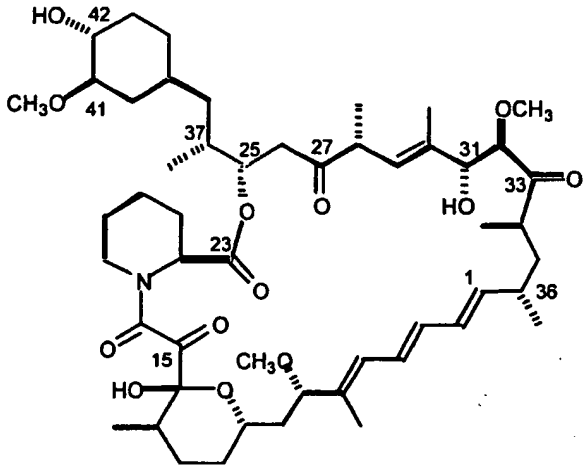
The present invention relates to novel chemical compounds having immunomodulatory activity and synthetic intermediates useful for the preparation of the novel compounds, and in particular to macrolide immunomodulators. More particularly, the invention relates to semisynthetic analogs of rapamycin, means for their preparation, pharmaceutical compositions containing such compounds, and methods of treatment employing the same.

Background of The Invention

The compound cyclosporine (cyclosporin A) has found wide use since its introduction in the fields of organ transplantation and immunomodulation, and has brought about a significant increase in the success rate for transplantation procedures. Recently, several classes of macrocyclic compounds having potent immunomodulatory activity have been discovered. Okuhara *et al.*, in European Patent Application No. 184,162, published June 11, 1986, disclose a number of macrocyclic compounds isolated from the genus *Streptomyces*, including the immunosuppressant FK-506, a 23-membered macrocyclic lactone, which was isolated from a strain of *S. tsukubaensis*.

Other related natural products, such as FR-900520 and FR-900523, which differ from FK-506 in their alkyl substituent at C-21, have been isolated from *S. hygroscopicus yakushimnaensis*. Another analog, FR-900525, produced by *S. tsukubaensis*, differs from FK-506 in the replacement of a pipecolic acid moiety with a proline group. Unsatisfactory side-effects associated with cyclosporine and FK-506 such as nephrotoxicity, have led to a continued search for immunosuppressant compounds having improved efficacy and safety, including an immunosupressive agent which is effective topically, but ineffective systemically (U.S. Patent No. 5,457,111).

Rapamycin is a macrocyclic triene antibiotic produced by *Streptomyces hygroscopicus*, which was found to have antifungal activity, particularly against *Candida albicans*, both in vitro and in vivo (C. Vezina et al., *J. Antibiot.* 1975, *28*, 721; S. N. Sehgal et al., *J. Antibiot.* 1975, *28*, 727; H. A. Baker et al., *J. Antibiot.* 1978, *31*, 539; U.S. Patent No. 3,929,992; and U.S. Patent No. 3,993,749).



Rapamycin

Rapamycin alone (U.S. Patent No. 4,885,171) or in combination with picibanil (U.S. Patent No. 4,401,653) has been shown to have antitumor activity. In 1977, rapamycin was also shown to be effective as an immunosuppressant in the experimental allergic encephalomyelitis model, a model for multiple sclerosis; in the adjuvant arthritis model, a model for rheumatoid arthritis; and was shown to effectively inhibit the formation of IgE-like antibodies (R. Martel et al., *Can. J. Physiol. Pharmacol.*, 1977, *55*, 48).

The immunosuppressive effects of rapamycin have also been disclosed in *FASEB*, 1989, *3*, 3411 as has its ability to prolong survival time of organ grafts in histoincompatible rodents (R. Morris, *Med. Sci. Res.*, 1989, *17*, 877). The ability of rapamycin to inhibit T-cell activation was disclosed by M. Strauch (*FASEB*, 1989, *3*, 3411). These and other biological effects of rapamycin are reviewed in *Transplantation Reviews*, 1992, *6*, 39-87.

Rapamycin has been shown to reduce neointimal proliferation in animal models, and to reduce the rate of restenosis in humans. Evidence has been published showing that rapamycin also exhibits an anti-inflammatory effect, a characteristic which supported its selection as an agent for the treatment of rheumatoid arthritis. Because both cell proliferation and inflammation are thought

to be causative factors in the formation of restenotic lesions after balloon angioplasty and stent placement, rapamycin and analogs thereof have been proposed for the prevention of restenosis.

Mono-ester and di-ester derivatives of rapamycin (esterification at positions 31 and 42) have been shown to be useful as antifungal agents (U.S. Patent No. 4,316,885) and as water soluble prodrugs of rapamycin (U.S. Patent No. 4,650,803).

Fermentation and purification of rapamycin and 30-demethoxy rapamycin have been described in the literature (C. Vezina et al. *J. Antibiot.* (Tokyo), 1975, *28* (10), 721; S. N. Sehgal et al., *J. Antibiot.* (Tokyo), 1975, *28*(10), 727; 1983, *36*(4), 351; N. L. Pavia et al., *J. Natural Products*, 1991, *54*(1), 167-177).

Numerous chemical modifications of rapamycin have been attempted. These include the preparation of mono- and di-ester derivatives of rapamycin (WO 92/05179), 27-oximes of rapamycin (EP0 467606); 42-oxo analog of rapamycin (U.S. Patent No. 5,023,262); bicyclic rapamycins (U.S. Patent No. 5,120,725); rapamycin dimers (U.S. Patent No. 5,120,727); silyl ethers of rapamycin (U.S. Patent No. 5,120,842); and arylsulfonates and sulfamates (U.S. Patent No. 5,177, 203). Rapamycin was recently synthesized in its naturally occuring enantiomeric form (K. C. Nicolaou et al., *J. Am. Chem. Soc.*, 1993, *115*, 4419-4420; S. L. Schreiber, *J. Am. Chem. Soc.*, 1993, *115*, 7906-7907; S. J. Danishefsky, *J. Am. Chem. Soc.*, 1993, *115*, 9345-9346.

It has been known that rapamycin, like FK-506, binds to FKBP-12 (Siekierka, J. J.; Hung, S. H. Y.; Poe, M.; Lin, C. S.; Sigal, N. H. *Nature*, **1989**, *341*, 755-757; Harding, M. W.; Galat, A.; Uehling, D. E.; Schreiber, S. L. *Nature* **1989**, *341*, 758-760; Dumont, F. J.; Melino, M. R.; Staruch, M. J.; Koprak, S. L.; Fischer, P. A.; Sigal, N. H. *J. Immunol.* **1990**, *144*, 1418-1424; Bierer, B. E.; Schreiber, S. L.; Burakoff, S. J. *Eur. J. Immunol.* **1991**, *21*, 439-445; Fretz, H.; Albers, M. W.; Galat, A.; Standaert, R. F.; Lane, W. S.; Burakoff, S. J.; Bierer, B. E.; Schreiber, S. L. *J. Am. Chem. Soc.* **1991**, *113*, 1409-1411). Recently it has been discovered that the rapamycin/FKBP-12 complex binds to yet another protein, which is distinct from calcineurin, the protein that the FK-506/FKBP-12 complex inhibits (Brown, E. J.; Albers, M. W.; Shin, T. B.; Ichikawa, K.; Keith, C. T.; Lane, W. S.; Schreiber, S. L. *Nature* **1994**, *369*, 756-758; Sabatini, D. M.; Erdjument-Bromage, H.; Lui, M.; Tempest, P.; Snyder, S. H. *Cell*, **1994**, 78, 35-43).

Percutaneous transluminal coronary angioplasty (PTCA) was developed by Andreas Gruntzig in the 1970's. The first canine coronary dilation was

performed on September 24, 1975; studies showing the use of PTCA were presented at the annual meetings of the American Heart Association the following year. Shortly thereafter, the first human patient was studied in Zurich, Switzerland, followed by the first American human patients in San Francisco and New York. While this procedure changed the practice of interventional cardiology with respect to treatment of patients with obstructive coronary artery disease, the procedure did not provide long-term solutions. Patients received only temporary abatement of the chest pain associated with vascular occlusion; repeat procedures were often necessary. It was determined that the existence of restenotic lesions severely limited the usefulness of the new procedure. In the late 1980's, stents were introduced to maintain vessel patency after angioplasty. Stenting is involved in 90% of angioplasty performed today. Before the introduction of stents, the rate of restenosis ranged from 30% to 50% of the patients who were treated with balloon angioplasty. The recurrence rate after dilatation of in-stent restenosis may be as high as 70% in selected patient subsets, while the angiographic restenosis rate in de novo stent placement is about 20%. Placement of the stent reduced the restenosis rate to 15% to 20%. This percentage likely represents the best results obtainable with purely mechanical stenting. The restenosis lesion is caused primarily by neointimal hyperplasia, which is distinctly different from atherosclerotic disease both in time-course and in histopathologic appearance. Restenosis is a healing process of damaged coronary arterial walls, with neointimal tissue impinging significantly on the vessel lumen. Vascular brachytherapy appears to be efficacious against in-stent restenosis lesions. Radiation, however, has limitations of practicality and expense, and lingering questions about safety and durability.

Accordingly, it is desired to reduce the rate of restenosis by at least 50% of its current level. It is for this reason that a major effort is underway by the interventional device community to fabricate and evaluate drug-eluting stents. Such devices could have many advantages if they were successful, principally since such systems would need no auxiliary therapies, either in the form of peri-procedural techniques or chronic oral pharmacotherapy.

Brief Description of the Drawings

Figure 1 shows blood concentrations ± SEM (n=3) of tetrazole-containing rapamycin analogs dosed in monkey.

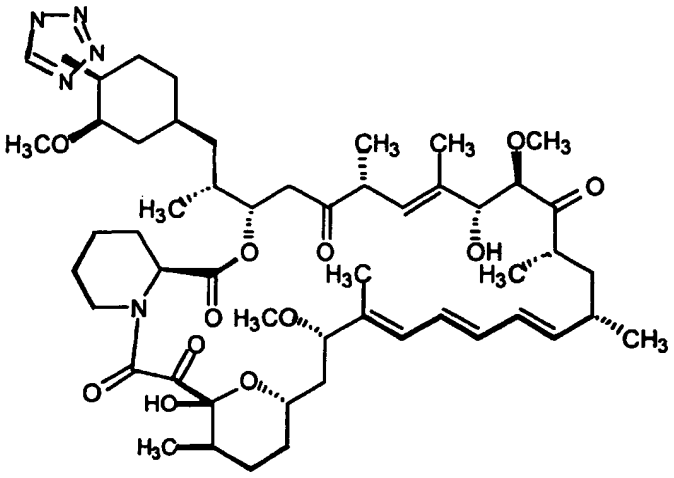
Figure 2 is a side view in elevation showing a stent suitable for use in this invention.

Figure 3A is a cross-sectional view of a vessel segment in which was placed a stent coated with a polymer only.

Figure 3B is a cross-sectional view of a vessel segment in which was placed a stent coated with a polymer plus drug.

Summary of the Invention

In one aspect of the present invention are disclosed compounds represented by the structural formula:



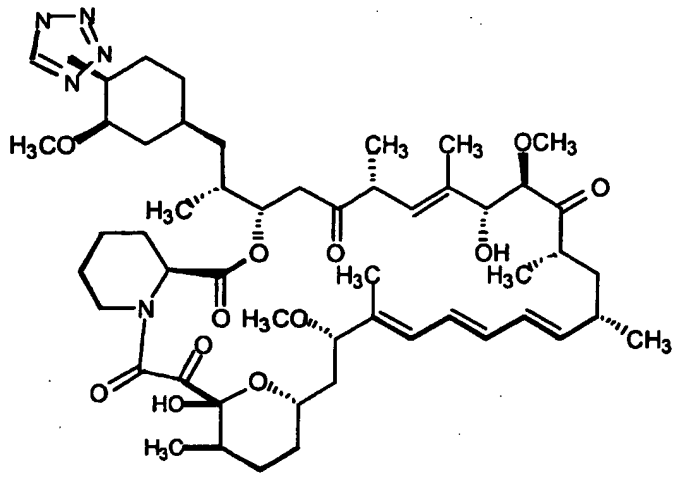
or a pharmaceutically acceptable salt or prodrug thereof.

Another object of the present invention is to provide synthetic processes for the preparation of such compounds from starting materials obtained by fermentation, as well as chemical intermediates useful in such synthetic processes.

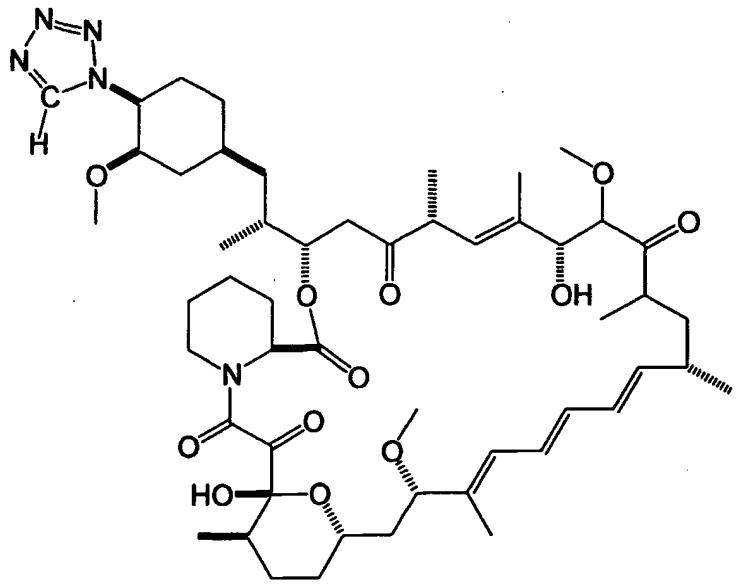
A further object of the invention is to provide pharmaceutical compositions containing, as an active ingredient, at least one of the above compounds.

Yet another object of the invention is to provide a method of treating a variety of disease states, including restenosis, post-transplant tissue rejection, immune and autoimmune dysfunction, fungal growth, and cancer.

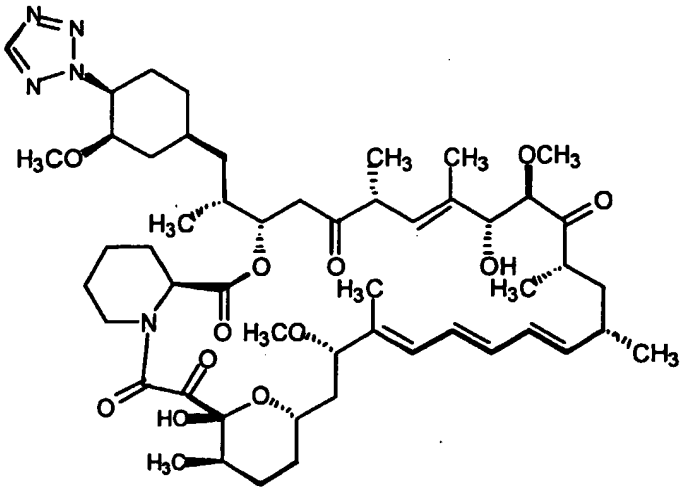
In another aspect this invention provides a medical device comprising a supporting structure having a coating on the surface thereof, the coating containing a therapeutic substance, such as, for example, a drug. Supporting structures for the medical devices that are suitable for use in this invention include, but are not limited to, coronary stents, peripheral stents, catheters, arterio-venous grafts, by-pass grafts, and drug delivery balloons used in the vasculature. Drugs that are suitable for use in this invention include, but are not limited to,



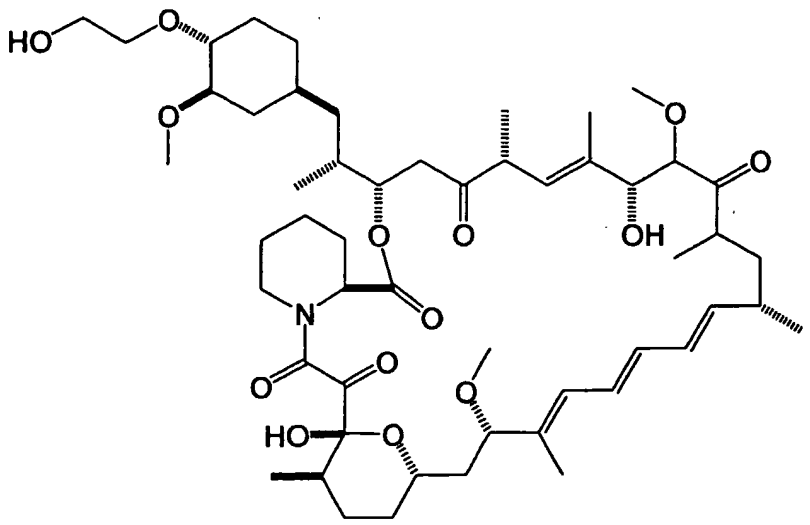
or a pharmaceutically acceptable salt or prodrug thereof, which includes



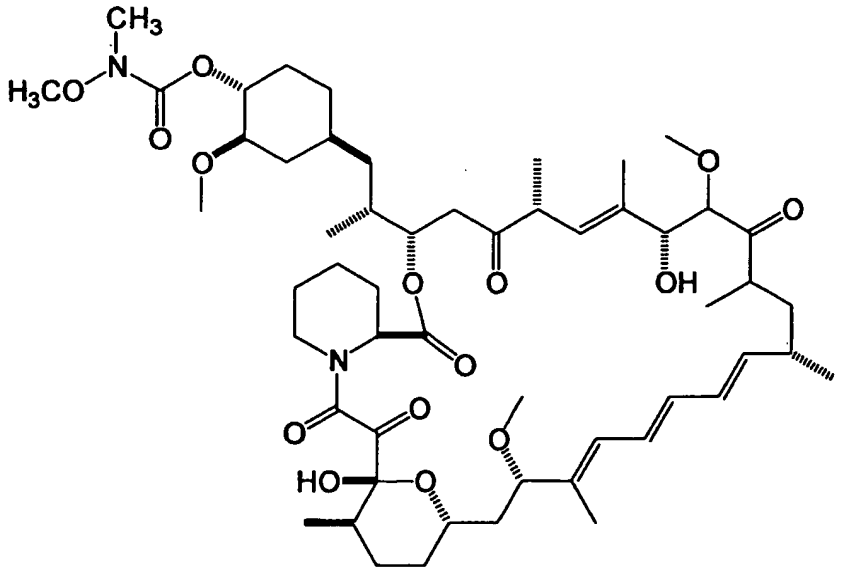
or a pharmaceutically acceptable salt or prodrug thereof, (hereinafter alternatively referred to as A-179578), and



or a pharmaceutically acceptable salt or prodrug thereof;



or a pharmaceutically acceptable salt or prodrug thereof, (hereinafter alternatively referred to as SDZ RAD or 40-O-(2-hydroxyethyl)-rapamycin);



or a pharmaceutically acceptable salt or prodrug thereof, (hereinafter alternatively referred to as A-94507).

Coatings that are suitable for use in this invention include, but are not limited to, polymeric coatings that can comprise any polymeric material in which the therapeutic agent, i. e., the drug, is substantially soluble. The coating can be hydrophilic, hydrophobic, biodegradable, or non-biodegradable. This medical

device reduces restenosis in vasculature. The direct coronary delivery of a drug such as A-179578 is expected to reduce the rate of restenosis to a level of about 0% to 25%.

Detailed Description of the Invention

Definition of Terms

The term "prodrug," as used herein, refers to compounds which are rapidly transformed in vivo to the parent compound of the above formula, for example, by hydrolysis in blood. A thorough discussion is provided by T. Higuchi and V. Stella, "Pro-drugs as Novel Delivery systems," Vol. 14 of the A. C. S. Symposium Series, and in Edward B. Roche, ed., "Bioreversible Carriers in Drug Design," American Pharmaceutical Association and Pergamon Press, 1987, both of which are incorporated herein by reference.

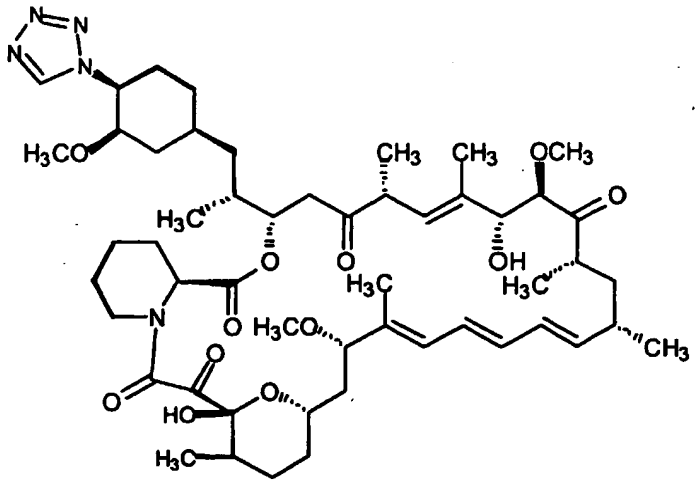
The term "pharmaceutically acceptable prodrugs", as used herein, refers to those prodrugs of the compounds of the present invention which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower mammals without undue toxicity, irritation, and allergic response, are commensurate with a reasonable benefit/risk ratio, and are effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention. Particularly preferred pharmaceutically acceptable prodrugs of this invention are prodrug esters of the C-31 hydroxyl group of compounds of this invention.

The term "prodrug esters," as used herein, refers to any of several ester-forming groups that are hydrolyzed under physiological conditions. Examples of prodrug ester groups include acetyl, ethanoyl, pivaloyl, pivaloyloxymethyl, acetoxymethyl, phthalidyl, methoxymethyl, indanyl, and the like, as well as ester groups derived from the coupling of naturally or unnaturally-occurring amino acids to the C-31 hydroxyl group of compounds of this invention.

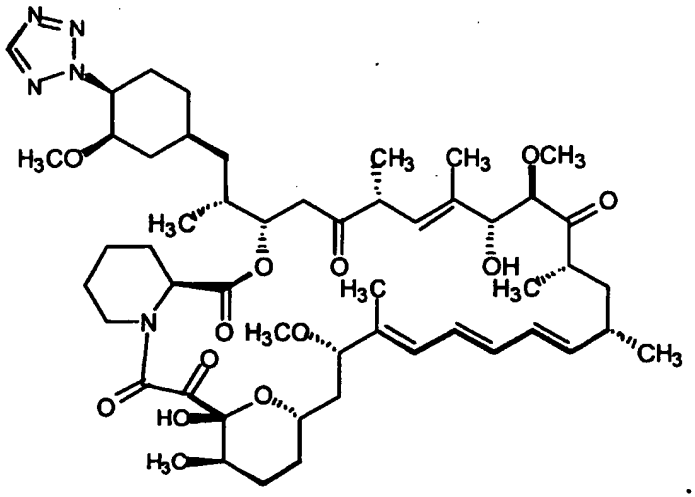
The term "supporting structure" means a framework that is capable of containing or supporting a pharmaceutically acceptable carrier or excipient, which carrier or excipient may contain a therapeutic agent, e.g., a drug or another compound. The supporting structure is typically formed of metal or a polymeric material.

Embodiments

In one embodiment of the invention is a compound of formula



In another embodiment of the invention is a compound of formula

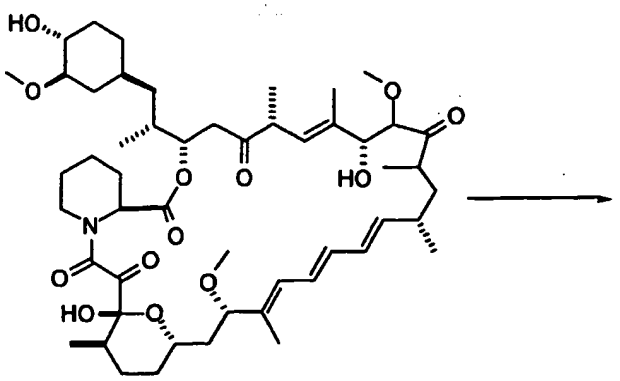


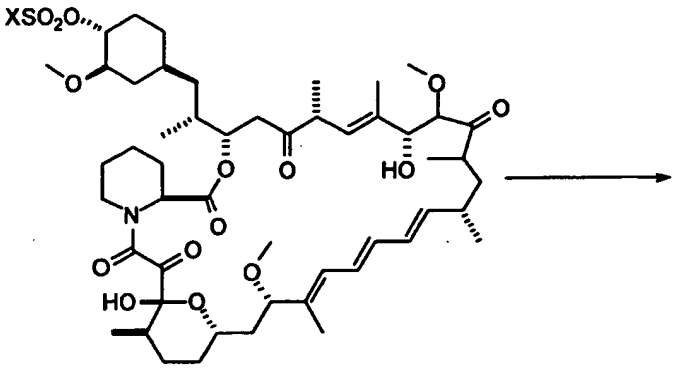
Preparation of Compounds of this Invention

The compounds and processes of the present invention will be better understood in connection with the following synthetic schemes which illustrate the methods by which the compounds of the invention may be prepared.

The compounds of this invention may be prepared by a variety of synthetic routes. A representative procedure is shown in Scheme 1.

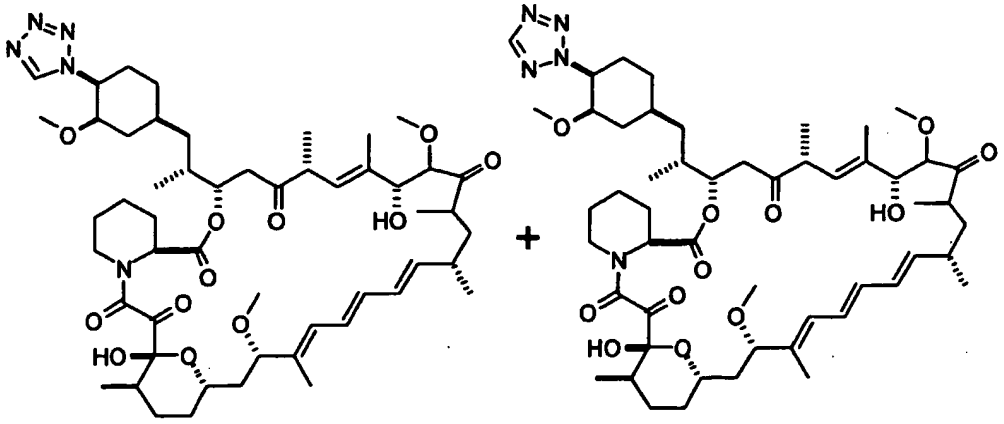
Scheme 1





X = F, CF_3

A



epimeric mixture (B/C)

As shown in Scheme 1, conversion of the C-42 hydroxyl of rapamycin to a trifluoromethanesulfonate or fluorosulfonate leaving group provided A. Displacement of the leaving group with tetrazole in the presence of a hindered, non-nucleophilic base, such as 2,6-lutidine, or, preferably, diisopropylethyl amine provided epimers B and C, which were separated and purified by flash column chromatography.

Synthetic Methods

The foregoing may be better understood by reference to the following examples which illustrate the methods by which the compounds of the invention may be prepared and are not intended to limit the scope of the invention as defined in the appended claims.

Example 1

42-Epi-(tetrazolyl)-rapamycin (less polar isomer)

Example 1A

A solution of rapamycin (100 mg, 0.11 mmol) in dichloromethane (0.6 mL) at -78 °C under a nitrogen atmosphere was treated sequentially with 2,6-lutidine (53 uL, 0.46 mmol, 4.3 eq.) and trifluoromethanesulfonic anhydride (37 uL, 0.22 mmol), and stirred thereafter for 15 minutes, warmed to room temperature and eluted through a pad of silica gel (6 mL) with diethyl ether. Fractions containing the triflate were pooled and concentrated to provide the designated compound as an amber foam.

Example 1B

42-Epi-(tetrazolyl)-rapamycin (less polar isomer)

A solution of Example 1A in isopropyl acetate (0.3 mL) was treated sequentially with diisopropylethylamine (87 □L, 0.5 mmol) and 1H-tetrazole (35 mg, 0.5 mmol), and thereafter stirred for 18 hours. This mixture was partitioned between water (10 mL) and ether (10 mL). The organics were washed with brine (10 mL) and dried (Na_2SO_4). Concentration of the organics provided a sticky yellow solid which was purified by chromatography on silica gel (3.5 g, 70-230 mesh) eluting with hexane (10 mL), hexane:ether (4:1(10 mL), 3:1(10 mL), 2:1(10 mL), 1:1(10 mL)), ether (30 mL), hexane:acetone (1:1(30mL)). One of the isomers was collected in the ether fractions.

MS (ESI) m/e 966 $(M)^-$;

Example 2

42-Epi-(tetrazolyl)-rapamycin (more polar isomer)

Example 2A

42-Epi-(tetrazolyl)-rapamycin (more polar isomer)

Collection of the slower moving band from the chromatography column using the hexane:acetone (1:1) mobile phase in Example 1B provided the designated compound.

MS (ESI) m/e 966 $(M)^-$.

In vitro Assay of Biological Activity

The immunosuppressant activity of the compounds of the present invention was compared to rapamycin and two rapamycin analogs: 40-epi-N-[2'-pyridone]-rapamycin and 40-epi-N-[4'-pyridone]-rapamycin, both disclosed in U. S. Patent No. 5,527,907. The activity was detemined using the human mixed lymphocyte reaction (MLR) assay described by Kino, T. *et al.* in *Transplantation Proceedings*, XIX(5):36-39, Suppl. 6 (**1987**). The results of the assay demonstrate that the compounds of the invention are effective immunomodulators at nanomolar concentrations, as shown in Table 1.

Table 1

Example	Human MLR IC_{50} ± S.E.M.(nM)
Rapamycin	0.91 ± 0.36
2-pyridone	12.39 ± 5.3
4-pyridone	0.43 ± 0.20
Example 1	1.70 ± 0.48
Example 2	0.66 ± 0.19

The pharmacokinetic behaviors of Example 1 and Example 2 were characterized following a single 2.5 mg/kg intravenous dose in cynomolgus monkey (n=3 per group). Each compound was prepared as 2.5 mg/mL solution in a 20% ethanol:30% propylene glycol:2% cremophor EL:48% dextrose 5% in water vehicle. The 1 mL/kg intravenous dose was administered as a slow bolus

(~1-2 minutes) in a saphenous vein of the monkeys. Blood samples were obtained from a femoral artery or vein of each animal prior to dosing and 0.1 (IV only), 0.25, 0.5, 1, 1.5, 2, 4, 6, 9, 12, 24, and 30 hours after dosing. The EDTA preserved samples were thoroughly mixed and extracted for subsequent analysis.

An aliquot of blood (1.0 mL) was hemolyzed with 20% methanol in water (0.5 ml) containing an internal standard. The hemolyzed samples were extracted with a mixture of ethyl acetate and hexane (1:1 (v/v), 6.0 mL). The organic layer was evaporated to dryness with a stream of nitrogen at room temperature. Samples were reconstituted in methanol: water (1:1, 150 µL). The title compounds (50 µL injection) were separated from contaminants using reverse phase HPLC with UV detection. Samples were kept cool (4 °C) through the run. All samples from each study were analyzed as a single batch on the HPLC.

Area under the curve (AUC) measurements of Example 1, Example 2 and the internal standard were determined using the Sciex MacQuan™ software. Calibration curves were derived from peak area ratio (parent drug/internal standard) of the spiked blood standards using least squares linear regression of the ratio versus the theoretical concentration. The methods were linear for both compounds over the range of the standard curve (correlation > 0.99) with an estimated quantitation limit of 0.1 ng/mL. The maximum blood concentration (C_{MAX}) and the time to reach the maximum blood concentration (T_{MAX}) were read directly from the observed blood concentration-time data. The blood concentration data were submitted to multi-exponential curve fitting using CSTRIP to obtain estimates of pharmacokinetic parameters. The estimated parameters were further defined using NONLIN84. The area under the blood concentration-time curve from 0 to t hours (last measurable blood concentration time point) after dosing (AUC_{0-t}) was calculated using the linear trapeziodal rule for the blood-time profiles. The residual area extrapolated to infinity, determined as the final measured blood concentration (C_t) divided by the terminal elimination rate constant (β), and added to AUC_{0-t} to produce the total area under the curve (AUC_{0-t}).

As shown in Figure 1 and Table 2, both Example 1 and Example 2 had a suprisingly substantially shorter terminal elimination half-life ($t_{1/2}$) when compared to rapamycin. Thus, only the compounds of the invention provide both sufficient efficacy (Table 1) and a shorter terminal half-life (Table 2).

Table 2

Compound	AUC ng·hr/mL	$t_{1/2}$ (hours)
Rapamycin	6.87	16.7
2-pyridone	2.55	2.8
4-pyridone	5.59	13.3
Example 1	2.35	5.0
Example 2	2.38	6.9

Methods of Treatment

The compounds of the invention, including but not limited to those specified in the examples, possess immunomodulatory activity in mammals (especially humans). As immunosuppressants, the compounds of the present invention are useful for the treatment and prevention of immune-mediated diseases such as the resistance by transplantation of organs or tissue such as heart, kidney, liver, medulla ossium, skin, cornea, lung, pancreas, intestinum tenue, limb, muscle, nerves, duodenum, small-bowel, pancreatic-islet-cell, and the like; graft-versus-host diseases brought about by medulla ossium transplantation; autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, Hashimoto's thyroiditis, multiple sclerosis, myasthenia gravis, type I diabetes, uveitis, allergic encephalomyelitis, glomerulonephritis, and the like. Further uses include the treatment and prophylaxis of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses, such as psoriasis, atopic dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeis dermatitis, lichen planus, pemphigus, bullous pemphigoid, epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, lupus erythematosus, acne and alopecia areata; various eye diseases (autoimmune and otherwise) such as keratoconjunctivitis, vernal conjunctivitis, uveitis associated with Behcet's disease, keratitis, herpetic keratitis, conical cornea, dystrophia epithelialis corneae, corneal leukoma, and ocular pemphigus. In addition reversible obstructive airway disease, which includes conditions such as asthma (for example, bronchial asthma, allergic asthma, intrinsic asthma, extrinsic asthma and dust asthma), particularly chronic or inveterate asthma (for example, late asthma and airway hyper-responsiveness), bronchitis, allergic

rhinitis, and the like are targeted by compounds of this invention. Inflammation of mucosa and blood vessels such as gastric ulcers, vascular damage caused by ischemic diseases and thrombosis. Moreover, hyperproliferative vascular diseases such as intimal smooth muscle cell hyperplasia, restenosis and vascular occlusion, particularly following biologically- or mechanically- mediated vascular injury, could be treated or prevented by the compounds of the invention. The compounds or drugs described herein can be applied to stents that have been coated with a polymeric compound. Incorporation of the compound or drug into the polymeric coating of the stent can be carried out by dipping the polymer-coated stent into a solution containing the compound or drug for a sufficient period of time (such as, for example, five minutes) and then drying the coated stent, preferably by means of air drying for a sufficient period of time (such as, for example, 30 minutes). The polymer-coated stent containing the compound or drug can then be delivered to the coronary vessel by deployment from a balloon catheter. In addition to stents, other devices that can be used to introduce the drugs of this invention to the vasculature include, but are not limited to grafts, catheters, and balloons. In addition, other compounds or drugs that can be used in lieu of the drugs of this invention include, but are not limited to, A-94507 and SDZ RAD). When used in the present invention, the coating can comprise any polymeric material in which the therapeutic agent, i. e., the drug, is substantially soluble. The purpose of the coating is to serve as a controlled release vehicle for the therapeutic agent or as a reservoir for a therapeutic agent to be delivered at the site of a lesion. The coating can be polymeric and can further be hydrophilic, hydrophobic, biodegradable, or non-biodegradable. The material for the polymeric coating can be selected from the group consisting of polycarboxylic acids, cellulosic polymers, gelatin, polyvinylpyrrolidone, maleic anhydride polymers, polyamides, polyvinyl alcohols, polyethylene oxides, glycosaminoglycans, polysaccharides, polyesters, polyurethanes, silicones, polyorthoesters, polyanhydrides, polycarbonates, polypropylenes, polylactic acids, polyglycolic acids, polycaprolactones, polyhydroxybutyrate valerates, polyacrylamides, polyethers, and mixtures and copolymers of the foregoing. Coatings prepared from polymeric dispersions such as polyurethane dispersions (BAYHYDROL, etc.) and acrylic acid latex dispersions can also be used with the therapeutic agents of the present invention.

Biodegradable polymers that can be used in this invention include polymers such as poly(L-lactic acid), poly(DL-lactic acid), polycaprolactone, poly(hydroxy butyrate), polyglycolide, poly(diaxanone), poly(hydroxy valerate),

polyorthoester; copolymers such as poly (lactide-co-glycolide), polyhydroxy(butyrate-co-valerate), polyglycolide-co-trimethylene carbonate; polyanhydrides; polyphosphoester; polyphosphoester-urethane; polyamino acids; polycyanoacrylates; biomolecules such as fibrin, fibrinogen, cellulose, starch, collagen and hyaluronic acid; and mixtures of the foregoing. Biostable materials that are suitable for use in this invention include polymers such as polyurethane, silicones, polyesters, polyolefins, polyamides, polycaprolactam, polyimide, polyvinyl chloride, polyvinyl methyl ether, polyvinyl alcohol, acrylic polymers and copolymers, polyacrylonitrile, polystyrene copolymers of vinyl monomers with olefins (such as styrene acrylonitrile copolymers, ethylene methyl methacrylate copolymers, ethylene vinyl acetate), polyethers, rayons, cellulosics (such as cellulose acetate, cellulose nitrate, cellulose propionate, etc.), parylene and derivatives thereof; and mixtures and copolymers of the foregoing.

Another polymer that can be used in this invention is poly(MPC_w:LAM_x:$HPMA_y$:$TSMA_z$) where w, x, y, and z represent the molar ratios of monomers used in the feed for preparing the polymer and MPC represents the unit 2-methacryoyloxyethylphosphorylcholine, LMA represents the unit lauryl methacrylate, HPMA represents the unit 2-hydroxypropyl methacrylate, and TSMA represents the unit 3-trimethoxysilylpropyl methacrylate. The drug-impregnated stent can be used to maintain patency of a coronary artery previously occluded by thrombus and/or atherosclerotic plaque. The delivery of an anti-proliferative agent reduces the rate of in-stent restenosis.

Other treatable conditions include but are not limited to ischemic bowel diseases, inflammatory bowel diseases, necrotizing enterocolitis, intestinal inflammations/allergies such as Coeliac diseases, proctitis, eosinophilic gastroenteritis, mastocytosis, Crohn's disease and ulcerative colitis; nervous diseases such as multiple myositis, Guillain-Barre syndrome, Meniere's disease, polyneuritis, multiple neuritis, mononeuritis and radiculopathy; endocrine diseases such as hyperthyroidism and Basedow's disease; hematic diseases such as pure red cell aplasia, aplastic anemia, hypoplastic anemia, idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia, agranulocytosis, pernicious anemia, megaloblastic anemia and anerythroplasia; bone diseases such as osteoporosis; respiratory diseases such as sarcoidosis, fibroid lung and idiopathic interstitial pneumonia; skin disease such as dermatomyositis, leukoderma vulgaris, ichthyosis vulgaris, photoallergic sensitivity and cutaneous T cell lymphoma; circulatory diseases such as arteriosclerosis, atherosclerosis, aortitis syndrome, polyarteritis nodosa and myocardosis; collagen diseases such as scleroderma,

Wegener's granuloma and Sjogren's syndrome; adiposis; eosinophilic fasciitis; periodontal disease such as lesions of gingiva, periodontium, alveolar bone and substantia ossea dentis; nephrotic syndrome such as glomerulonephritis; male pattern aleopecia or alopecia senilis by preventing epilation or providing hair germination and/or promoting hair generation and hair growth; muscular dystrophy; Pyoderma and Sezary's syndrome; Addison's disease; active oxygen-mediated diseases, as for example organ injury such as ischemia-reperfusion injury of organs (such as heart, liver, kidney and digestive tract) which occurs upon preservation, transplantation or ischemic disease (for example, thrombosis and cardiac infarction); intestinal diseases such as endotoxin-shock, pseudomembranous colitis and colitis caused by drug or radiation; renal diseases such as ischemic acute renal insufficiency and chronic renal insufficiency; pulmonary diseases such as toxinosis caused by lung-oxygen or drug (for example, paracort and bleomycins), lung cancer and pulmonary emphysema; ocular diseases such as cataracta, siderosis, retinitis, pigmentosa, senile macular degeneration, vitreal scarring and corneal alkali burn; dermatitis such as erythema multiforme, linear IgA ballous dermatitis and cement dermatitis; and others such as gingivitis, periodontitis, sepsis, pancreatitis, diseases caused by environmental pollution (for example, air pollution), aging, carcinogenesis, metastasis of carcinoma and hypobaropathy; diseases caused by histamine or leukotriene-C_4 release; Behcet's disease such as intestinal-, vasculo- or neuro-Behcet's disease, and also Behcet's which affects the oral cavity, skin, eye, vulva, articulation, epididymis, lung, kidney and so on. Furthermore, the compounds of the invention are useful for the treatment and prevention of hepatic disease such as immunogenic diseases (for example, chronic autoimmune liver diseases such as autoimmune hepatitis, primary biliary cirrhosis and sclerosing cholangitis), partial liver resection, acute liver necrosis (e.g. necrosis caused by toxin, viral hepatitis, shock or anoxia), B-virus hepatitis, non-A/non-B hepatitis, cirrhosis (such as alcoholic cirrhosis) and hepatic failure such as fulminant hepatic failure, late-onset hepatic failure and "acute-on-chronic" liver failure (acute liver failure on chronic liver diseases), and moreover are useful for various diseases because of their useful activity such as augmention of chemotherapeutic effect, cytomegalovirus infection, particularly HCMV infection, anti-inflammatory activity, sclerosing and fibrotic diseases such as nephrosis, scleroderma, pulmonary fibrosis, arteriosclerosis, congestive heart failure, ventricular hypertrophy, post-surgical adhesions and scarring, stroke, myocardial infarction and injury associated with ischemia and reperfusion, and the like.

Additionally, compounds of the invention possess FK-506 antagonistic properties. The compounds of the present invention may thus be used in the treatment of immunodepression or a disorder involving immunodepression. Examples of disorders involving immunodepression include AIDS, cancer, fungal infections, senile dementia, trauma (including wound healing, surgery and shock) chronic bacterial infection, and certain central nervous system disorders. The immunodepression to be treated may be caused by an overdose of an immunosuppressive macrocyclic compound, for example derivatives of 12-(2-cyclohexyl-1-methylvinyl)-13, 19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0 ^{4,9}] octacos-18-ene such as FK-506 or rapamycin. The overdosing of such medicants by patients is quite common upon their realizing that they have forgotten to take their medication at the prescribed time and can lead to serious side effects.

The ability of the compounds of the invention to treat proliferative diseases can be demonstrated according to the methods described in Bunchman ET and CA Brookshire, Transplantation Proceed. 23 967-968 (1991); Yamagishi, et al., Biochem. Biophys. Res. Comm. 191 840-846 (1993); and Shichiri, et al., J. Clin. Invest. 87 1867-1871 (1991). Proliferative diseases include smooth muscle proliferation, systemic sclerosis, cirrhosis of the liver, adult respiratory distress syndrome, idiopathic cardiomyopathy, lupus erythematosus, diabetic retinopathy or other retinopathies, psoriasis, scleroderma, prostatic hyperplasia, cardiac hyperplasia, restenosis following arterial injury or other pathologic stenosis of blood vessels. In addition, these compounds antagonize cellular responses to several growth factors, and therefore possess antiangiogenic properties, making them useful agents to control or reverse the growth of certain tumors, as well as fibrotic diseases of the lung, liver, and kidney.

Aqueous liquid compositions of the present invention are particularly useful for the treatment and prevention of various diseases of the eye such as autoimmune diseases (including, for example, conical cornea, keratitis, dysophia epithelialis corneae, leukoma, Mooren's ulcer, sclevitis and Graves' ophthalmopathy) and rejection of corneal transplantation.

When used in the above or other treatments, a therapeutically effective amount of one of the compounds of the present invention may be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt, ester or prodrug form. Alternatively, the compound may be administered as a pharmaceutical composition containing the compound of interest in combination

with one or more pharmaceutically acceptable excipients. The phrase "therapeutically effective amount" of the compound of the invention means a sufficient amount of the compound to treat disorders, at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgement. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

The total daily dose of the compounds of this invention administered to a human or lower animal may range from about 0.01 to about 10 mg/kg/day. For purposes of oral administration, more preferable doses may be in the range of from about 0.001 to about 3 mg/kg/day. For the purposes of local delivery from a stent, the daily dose that a patient will receive depends on the length of the stent. For example, a 15 mm coronary stent may contain a drug in an amount ranging from about 1 to about 120 micrograms and may deliver that drug over a time period ranging from several hours to several weeks. If desired, the effective daily dose may be divided into multiple doses for purposes of administration; consequently, single dose compositions may contain such amounts or submultiples thereof to make up the daily dose. Topical adminstration may involve doses ranging from 0.001 to 3% mg/kg/day, depending on the site of application.

Pharmaceutical Compositions

The pharmaceutical compositions of the present invention comprise a compound of the invention and a pharmaceutically acceptable carrier or excipient, which may be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, as an oral or nasal spray, or locally, as in a stent

placed within the vasculature. The phrase "pharmaceutically acceptable carrier" means a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral," as used herein, refers to modes of administration which include intravenous, intraarterial, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection, infusion, and placement, such as , for example, in vasculature.

Pharmaceutical compositions of this invention for parenteral injection comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), carboxymethylcellulose and suitable mixtures thereof, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents that delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of the drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other

biodegradable polymers include poly(orthoesters) and poly(anhydrides) Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft, semi-solid and hard-filled gelatin capsules or liquid-filled capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. Those embedding compositions containing a drug can be placed on medical devices, such as stents, grafts, catheters, and balloons.

The active compounds can also be in micro-encapsulated form, if appropriate, with one or more of the above-mentioned excipients.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, and tragacanth, and mixtures thereof.

Topical administration includes administration to the skin or mucosa, including surfaces of the lung and eye. Compositions for topical administration, including those for inhalation, may be prepared as a dry powder which may be pressurized or non-pressurized. In non-pressurized powder compositions, the active ingredient in finely divided form may be used in admixture with a larger-sized pharmaceutically acceptable inert carrier comprising particles having a size, for example, of up to 100 micrometers in diameter. Suitable inert carriers include sugars such as lactose. Desirably, at least 95% by weight of the particles of the active ingredient have an effective particle size in the range of 0.01 to 10 micrometers. Compositions for topical use on the skin also include oinments, creams, lotions, and gels.

Alternatively, the composition may be pressurized and contain a compressed gas, such as nitrogen or a liquified gas propellant. The liquified propellant medium and indeed the total composition is preferably such that the active ingredient does not dissolve therein to any substantial extent. The pressurized composition may also contain a surface active agent. The surface active agent may be a liquid or solid non-ionic surface active agent or may be a solid anionic surface active agent. It is preferred to use the solid anionic surface active agent in the form of a sodium salt.

A further form of topical administration is to the eye, as for the treatment of immune-mediated conditions of the eye such as automimmue diseases, allergic

or inflammatory conditions, and corneal transplants. The compound of the invention is delivered in a pharmaceutically acceptable ophthalmic vehicle, such that the compound is maintained in contact with the ocular surface for a sufficient time period to allow the compound to penetrate the corneal and internal regions of the eye, as for example the anterior chamber, posterior chamber, vitreous body, aqueous humor, vitreous humor, cornea, iris/cilary, lens, choroid/retina and sclera. The pharmaceutically acceptable ophthalmic vehicle may, for example, be an ointment, vegetable oil or an encapsulating material.

Compositions for rectal or vaginal administration are preferably suppositories or retention enemas which can be prepared by mixing the compounds of this invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at room temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

Compounds of the present invention can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to a compound of the present invention, stabilizers, preservatives, excipients, and the like. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art. See, for example, Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (l976), p. 33 *et seq*.

Compounds of the present invention may also be coadministered with one or more immunosuppressant agents. The immunosuppressant agents within the scope of this invention include, but are not limited to, IMURAN® azathioprine sodium, brequinar sodium, SPANIDIN® gusperimus trihydrochloride (also known as deoxyspergualin), mizoribine (also known as bredinin), CELLCEPT® mycophenolate mofetil, NEORAL® Cylosporin A (also marketed as different formulation of Cyclosporin A under the trademark SANDIMMUNE®), PROGRAF® tacrolimus (also known as FK-506), sirolimus and RAPAMUNE®, leflunomide (also known as HWA-486), glucocorticoids, such as prednisolone and its

derivatives, antibody therapies such as orthoclone (OKT3) and Zenapax®, and antithymyocyte globulins, such as thymoglobulins.

Example 3

The purpose of this example was to determine the effects of a rapamycin analog on neointimal formation in porcine coronary arteries containing stents. This example illustrates that the rapamycin analog A-179578, when compounded and delivered from the Biocompatibles BiodiviYsio PC Coronary stent favorably affects neointimal hyperplasia and lumen size in porcine coronary arteries. This finding suggests that such a combination may be of substantial clinical benefit if properly applied in humans by limiting neointimal hyperplasia.

The agent A-179578 is a rapamycin analog. The study set forth in this example was designed to assess the ability of the rapamycin analog A-179578 to reduce neointimal hyperplasia in a porcine coronary stent model. Efficacy of A-179578 in this model would suggest its clinical potential for the limitation and treatment of coronary restenosis in stents following percutaneous revascularization. The domestic swine was used because this model appears to yield results comparable to other investigations seeking to limit neointimal hyperplasia in human subjects.

The example tested A-179578 eluted from coronary stents placed in juvenile farm pigs, and compared these results with control stents. The control stents had polymer alone covering its struts. This is important, for the polymer itself must not stimulate neointimal hyperplasia to a substantial degree. As the eluted drug disappears, an inflammatory response to the polymer could conceivably result in a late "catch-up phenomenon" where the restenosis process is not stopped, but instead slowed. This phenomenon would result in restenosis at late dates in human subjects.

Stents were implanted in two blood vessels in each pig. Pigs used in this model were generally 2-4 months old and weighed 30-40 Kg. Two coronary stents were thus implanted in each pig by visually assessing a "normal" stent:artery ratio of 1.1-1.2.

Beginning on the day of the procedure, pigs were given oral aspirin (325 mg daily) and continued for the remainder of their course. General anesthesia was achieved by means of intramuscular injection followed by intravenous ketamine (30 mg/kg) and xylazine (3 mg/kg). Additional medication at the time of induction included atropine (1 mg) and flocillin (1 g) administered intramuscularly.

During the stenting procedure, an intraarterial bolus of 10,000 units of heparin was administered.

Arterial access was obtained by cutdown on the right external carotid and placement of an 8F sheath. After the procedure, the animals were maintained on a normal diet without cholesterol or other special supplementation.

The BiodivYsio stent was used with nominal vessel target size of 3.0 mm. See Figure 2. Two coronary arteries per pig were assigned at random to deployment of the stents. The stent was either a drug eluting stent (polymer plus drug stent) or a stent coated with a polymer only (polymer only stent). The stents were delivered by means of standard guide catheters and wires. The stent balloons were inflated to appropriate sizes for less than 30 seconds.

Each pig had one polymer only stent and one polymer plus drug stent placed in separate coronary arteries, so that each pig would have one stent for drug and one for control.

A sample size of 20 pigs total was chosen to detect a projected difference in neointimal thickness of 0.2 mm with a standard deviation of 0.15 mm, at a power of 0.95 and beta 0.02.

Animals were euthanized at 28 days for histopathologic examination and quantification. Following removal of the heart from the perfusion pump system, the left atrial appendage was removed for access to the proximal coronary arteries. Coronary arterial segments with injuries were dissected free of the epicardium. Segments containing lesions was isolated, thereby allowing sufficient tissue to contain uninvolved blood vessel at either end. The foregoing segments, each roughly 2.5 cm in length, were embedded and processed by means of standard plastic embedding techniques. The tissues were subsequently processed and stained with hematoxylin-eosin and elastic-van Gieson techniques.

Low and high power light microscopy were used to make length measurements in the plane of microscopic view by means of a calibrated reticle and a digital microscopy system connected to a computer employing calibrated analysis software.

The severity of vessel injury and the neointimal response were measured by calibrated digital microscopy. The importance of the integrity of the internal elastic lamina is well-known to those skilled in the art. A histopathologic injury score in stented blood vessels has been validated as being closely related to neointimal thickness. This score is related to depth of injury and is as follows:

Score	Description of Injury
0	Internal elastic lamina intact; endothelium typically denuded, media compressed but not lacerated.
1	Internal elastic lamina lacerated; media typically compressed but not lacerated.
2	Internal elastic lacerated; media visibly lacerated; external elastic lamina intact but compressed.
3	External elastic lamina lacerated; typically large lacerations of media extending through the external elastic lamina; coil wires sometimes residing in adventitia.

This quantitative measurement of injury was assessed for all stent wires of each stent section. The calibrated digital image was also used to measure at each stent wire site the neointimal thickness. Lumen area, area contained with the internal elastic lamina, and area within the external elastic lamina were also measured.

At each stent wire site for a given section, the neointimal thickness was averaged to obtain a mean injury score for each section. The measurement of neointimal thickness was made to the abluminal side of the stent wire, because the neointimal in all cases includes this thickness.

The mid-stent segment was used for measurement, analysis, and comparison. Data were also recorded (and included in the data section of this report) for proximal and distal segments.

The data analysis methods for this study did not need to take into account variable arterial injury across treatment/control groups, because mild to moderate injury is sensitive enough to detect treatment differences. Paired t-testing was performed to compare variables across the polymer only stents (control group) and polymer plus drug stents (treatment group). No animal died in this study before scheduled timepoints.

Table 3 shows the pigs and arteries used. In Table 3, LCX means the circumflex branch of the left coronary artery, LAD means the left anterior descending coronary artery, and RCA means the right coronary artery.

Table 3

Pigs and Vessels Used

1	2000-G-693	RCA - Control
	2000-G-693	LCX - Test
2	2000-G-698	RCA - Test
	2000-G-698	LAD - Control
3	2000-G-702	RCA - Test
	2000-G-702	LAD - Control
4	2000-G-709	RCA - Control
	2000-G-709	LAD - Test
5	2000-G-306	RCA - Control
	2000-G-306	LAD - Test
	2000-G-306	* LCX - Test
6	2000-G-672	RCA - Test
	2000-G-672	LAD - Control
7	2000-G-712	RCA - Control
	2000-G-712	LCX - Test
8	2000-G-735	RCA - Control
	2000-G-735	LAD - Test
9	2000-G-736	RCA - Control
	2000-G-736	LCX - Test
10	2000-G-740	RCA - Test
	2000-G-740	LAD - Control
11	2000-G-742	LAD - Test
	2000-G-742	OM (LCX) - Control
12	2000-G-744	RCA - Test
	2000-G-744	LAD - Control
13	2000-G-748	RCA - Test
	2000-G-748	LAD - Control
14	2000-G-749	RCA - Control
	2000-G-749	LCX - Test
15	2000-G-753	RCA - Control
	2000-G-753	LAD - Test
16	2000-G-754	RCA - Test
	2000-G-754	LCX -Control
17	2000-G-755	RCA - Control
	2000-G-755	LAD - Test
18	2000-G-756	RCA - Test
	2000-G-756	LAD - Control
19	2000-G-757	LAD - Control
	2000-G-757	LCX - Test
20	2000-G-760	LAD - Test
	2000-G-760	LCX -Control

Table 4 shows the summary results for all data for mean injury and neointimal thickness for each stent, including proximal, mid, and distal segments.

Table 4 also shows lumen size, percent stenosis, and artery size as measured by the internal elastic laminae (IEL) and external elastic laminae (EEL).

Table 4

Summary: All Measures (Distal, Mid, Proximal)

ID	prox ref	dist ref	lumen	IEL	EEL	mean injury	% stenosis	Neointimal area	NIT
Control	Distal								
Mean	4.46	3.96	4.88	7.66	9.00	0.22	36.10	2.79	0.41
SD	1.20	1.16	1.30	1.15	1.10	0.26	15.41	1.29	0.17
Control	Mid								
Mean	4.46	3.96	4.94	7.71	9.08	0.08	36.23	2.77	0.38
SD	1.20	1.16	1.44	1.07	1.15	0.14	14.93	1.20	0.16
Control	Proximal								
Mean	4.46	3.96	5.11	7.89	9.30	0.15	35.35	2.78	0.38
SD	1.20	1.16	1.38	1.33	1.42	0.22	11.94	1.04	0.12
Test	Distal								
Mean	4.26	3.41	6.04	7.70	9.01	0.26	22.35	1.66	0.25
SD	1.26	0.96	1.55	1.49	1.47	0.43	8.58	0.58	0.06
Test	Mid								
Mean	4.26	3.41	6.35	7.75	8.98	0.04	18.71	1.41	0.22
SD	1.26	0.96	1.29	1.18	1.31	0.07	5.68	0.33	0.05
Test	Proximal								
Mean	2.56	2.15	3.31	4.06	4.66	0.19	16.79	1.29	0.18
SD	1.66	1.37	2.39	3.48	4.15	0.13	9.97	0.80	0.12

There was no statistically significant difference for neointimal area or thickness across proximal, mid, or distal segments within the test group (polymer plus drug stents) or control groups (polymer only stents). This observation is quite consistent with prior studies, and thus allows use of only the mid segment for statistical comparison of test devices (polymer plus drug stents) vs. control devices (polymer only stents).

Table 5 shows the statistical t-test comparisons across test groups and control groups. There was a statistically significant difference in neointimal thickness, neointimal area, lumen size, and percent lumen stenosis, the drug eluting stent being clearly favored. Conversely, there were no statistically significant differences between the test group (polymer plus drug stents) and the

control group (polymer only stents) for mean injury score, external elastic laminae, or internal elastic laminae areas.

Table 5

Statistical Comparison of Test vs. Control Parameters: Mid-Section Data

t-test Statistics							
Parameter	Difference	t-test	DF	Std Error	Lower 95%	Upper 95%	p
Lumen	-1.17	-2.28	38	0.52	-2.21	-0.13	0.029
IEL	0.03	0.088	38	0.36	-0.71	0.78	0.93
EEL	0.2	0.499	38	0.39	-0.599	0.99	0.62
NI Thickness	0.18	5.153	38	0.034	0.106	0.244	<.0001
NI Area	1.21	3.62	38	0.33	0.53	1.88	0.0008
Mean Injury	0.038	1.137	38	0.033	-0.02	0.106	0.26
% Stenosis	14.54	2.97	38	4.9	4.61	24.47	0.005

The reference arteries proximal and distal to the stented segments were observed, and quantitated. These vessels appeared normal in all cases, uninjured in both the control group (polymer only stents) and the test group (polymer plus drug stents). See Figures 3A and 3B. The data below show there were no statistically significant differences in size between the stents in the control group and the stents in the test group.

	Proximal Reference Diameter (mm)	Distal Reference Diameter (mm)
Control (mean ± SD)	4.46 ± 1.20	3.96 ± 1.16
Test (mean + SD)	4.26 ± 1.26	3.41 ± 0.96

The data suggest that statistically significant differences exist, and these differences favor the stent that elutes A-179578. The stent of this invention results in lower neointimal area, lower neointimal thickness, and greater lumen area. There were no significant differences within the test group (polymer plus drug stents) and the control group (polymer only stents) for neointimal or injury parameters. There were no significant differences in artery sizes (including the stent) for the control group compared to the test group. These latter findings suggest no significant difference in the arterial remodeling characteristics of the polymeric coating containing the drug.

At most, mild inflammation was found on both the polymer plus drug stent and the polymer only stent. This finding suggests that the polymer exhibits satisfactory biocompatibility, even without drug loading. Other studies show that when drug has completely gone from the polymer, the polymer itself creates enough inflammation to cause neointima. This phenomenon may be responsible for the late □catch-up□ phenomenon of clinical late restenosis. Because the polymer in this example did not cause inflammation in the coronary arteries, late problems related to the polymer after the drug is exhausted are unlikely.

In conclusion, a stent containing the compound A-179578 with a polymer showed a reduction in neointimal hyperplasia in the porcine model when placed in a coronary artery.

Example 4

The purpose of this example is to determine the rate of release of the A-179578 drug from 316L Electropolished Stainless Steel Coupons coated with a biocompatible polymer containing phosphorylcholine side groups.

Rubber septa from lids from HPLC vials were removed from the vials and placed into glass vials so that the "Teflon" side faced up. These septa served as supports for the test samples. The test samples were 316L stainless steel coupons that had been previously coated with a biocompatible polymer containing phosphorylcholine side groups (PC polymer). Coronary stents are commonly made of 316L stainless steel and can be coated with the PC polymer to provide a depot site for loading drugs. The coated coupons, which serve to simulate stents, were placed onto the septa. By using a glass Hamilton Syringe, a solution of A-179578 and ethanol (10 μl) was applied to the surface of each coupon. The solution contained A-179578 (30.6 mg) dissolved in 100% ethanol

(3.0 ml). The syringe was cleaned with ethanol between each application. The cap to the glass vial was placed on the vial loosely, thereby assuring proper ventilation. The coupon was allowed to dry for a minimum of 1.5 hours. Twelve (12) coupons were loaded in this way - six being used to determine the average amount of drug loaded onto the device and six being used to measure the time needed to release the drug from the devices.

To determine the total amount of A-179578 loaded onto a coupon, a coupon was removed from the vial and placed into 50/50 acetonitrile/ 0.01M phosphate buffer (pH 6.0, 5.0 ml). The coupon was placed onto a 5210 Branson sonicator for one hour. The coupon was then removed from the solution, and the solution was assayed by HPLC.

The time release studies were performed by immersing and removing the individual coupons from fresh aliquots (10.0 ml) of 0.01 M phosphate buffer at a pH of 6.0 at each of the following time intervals - 5, 15, 30 and 60 minutes. For the remaining time points of 120, 180, 240, 300, 360 minutes, volumes of 5.0 ml of buffer were used. To facilitate mixing during the drug release phase, the samples were placed onto an Eberbach shaker set at low speed. All solution aliquots were assayed by HPLC after the testing of the last sample was completed.

The HPLC analysis was performed with a Hewlett Packard series 1100 instrument having the following settings:

Injection Volume	=	100 µl
Acquisition Time	=	40 minutes
Flow Rate	=	1.0 ml/min
Column Temperature	=	40 °C
Wavelength	=	278 nm
Mobile Phase	=	65% Acetonitrile/35% H_2O
Column	=	YMC ODS-A S5 µm,4.6 x 250mm Part No. A12052546WT

The results from the above experiment showed the following release data:

Table 6

Time (min.)	Percent Release	Standard Deviation
0.00	0.00	0.00
5.00	1.87	1.12
15.00	2.97	1.47
30.00	3.24	1.28
60.00	3.29	1.29
120.00	3.92	1.28
180.00	4.36	1.33
240.00	4.37	1.35
300.00	6.34	2.07
360.00	7.88	1.01

Example 5

The purpose of this example was to determine the loading and release of A-179578 from 15mm BiodivYsio drug delivery stents.

To load the stents with drug, a solution of A-179578 in ethanol at a concentration of 50 mg/ml was prepared and dispensed into twelve vials. Twelve individual polymer-coated stents were placed on fixtures designed to hold the stent in a vertical position and the stents were immersed vertically in the drug solution for five minutes. The stents and fixtures were removed from the vials and excess drug solution was blotted away by contacting the stents with an absorbant material. The stents were then allowed to dry in air for 30 minutes in an inverted vertical position.

The stents were removed from the fixtures, and each stent was placed into 50/50 acetonitrile/phosphate buffer (pH 5.1, 2.0 ml) and sonicated for one hour. The stents were removed from the solution and solutions were assayed for concentration of drug, which allowed calculation of the amount of drug originally on the stents. This method was independently shown to remove at least 95% of the drug from the stent coating. On average, the stents contained 60 micrograms of drug ± 20 micrograms.

The drug-loaded stents were placed on the fixtures and placed into 0.01 M phosphate buffer (pH = 6.0, 1.9 ml) in individual vials. These samples were placed onto an Eberbach shaker set at low speed to provide back-and-forth agitation. To avoid approaching drug saturation in the buffer, the stents were transferred periodically to fresh buffer vials at the following points: 15, 30, 45, 60, 120, 135, 150, 165, 180, 240, 390 minutes. The dissolution buffer vials were

assayed by HPLC for the drug concentration at the end of the drug release period studied. The data, represented as % cumulative release of the drug as a function of time, is shown in tabular form below:

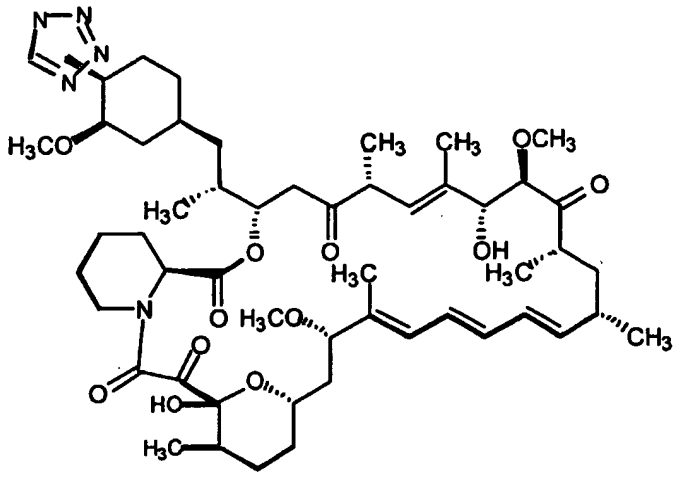
Table 7

Time (min)	% Cumulative Release of Drug
15	0.3
30	1.1
45	2.1
60	3.2
120	4.3
135	5.9
150	6.3
165	6.8
180	7.4
240	10.8
390	13.2

It is understood that the foregoing detailed description and accompanying examples are merely illustrative and are not to be taken as limitations upon the scope of the invention, which is defined solely by the appended claims and their equivalents. Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications, including without limitation those relating to the chemical structures, substituents, derivatives, intermediates, syntheses, formulations and/or methods of use of the invention, may be made without departing from the spirit and scope thereof.

WHAT IS CLAIMED IS:

1. A medical device comprising a supporting structure and the therapeutic substance



or a pharmaceutically acceptable salt or prodrug thereof.

2. The medical device of claim 1, wherein said supporting structure is selected from the group consisting of coronary stents, peripheral stents, catheters, arterio-venous grafts, by-pass grafts, and drug delivery balloons used in the vasculature.

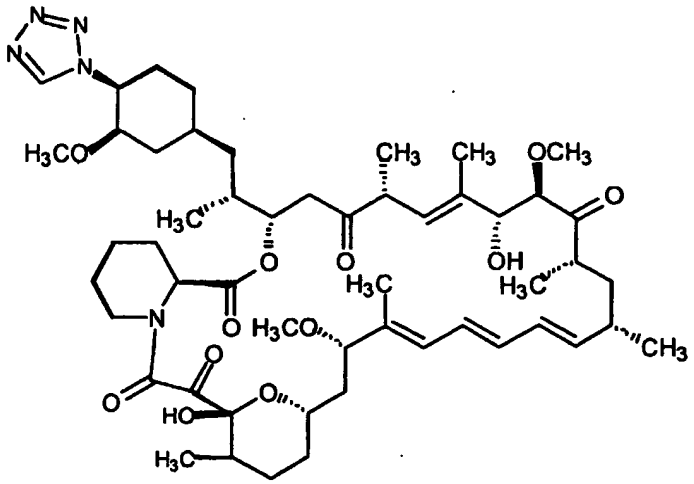
3. The medical device of claim 1, wherein said supporting structure further comprises a coating, said coating containing said therapeutic substance.

4. The medical device of claim 3, wherein said coating is polymeric.

5. The medical device of claim 4, wherein said polymeric coating is biostable.

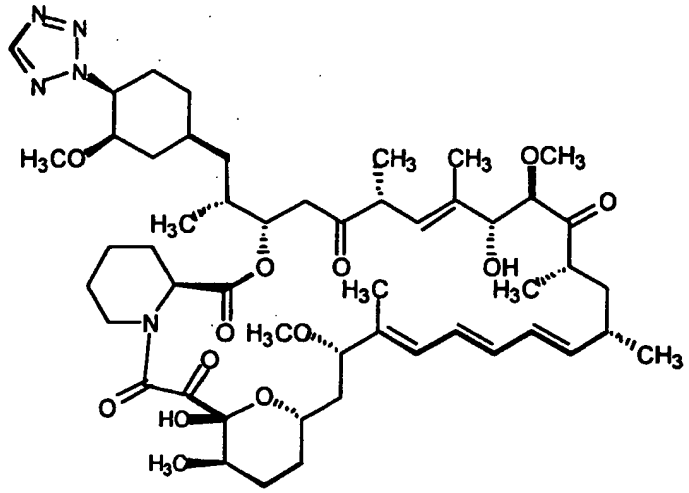
6. The medical device of claim 4, wherein said polymeric coating is biodegradable.

7. The medical device of claim 1, wherein said therapeutic substance is



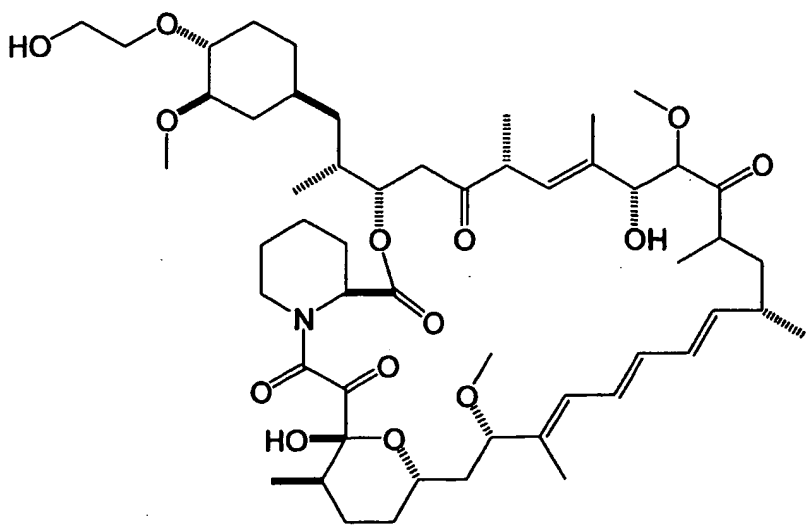
or a pharmaceutically acceptable salt or prodrug thereof.

8. The medical device of claim 1, wherein said therapeutic substance is



or a pharmaceutically acceptable salt or prodrug thereof.

9. A medical device comprising a supporting structure and the therapeutic substance



or a pharmaceutically acceptable salt or prodrug thereof.

10. The medical device of claim 9, wherein said supporting structure is selected from the group consisting of coronary stents, peripheral stents, catheters, arterio-venous grafts, by-pass grafts, and drug delivery balloons used in the vasculature.

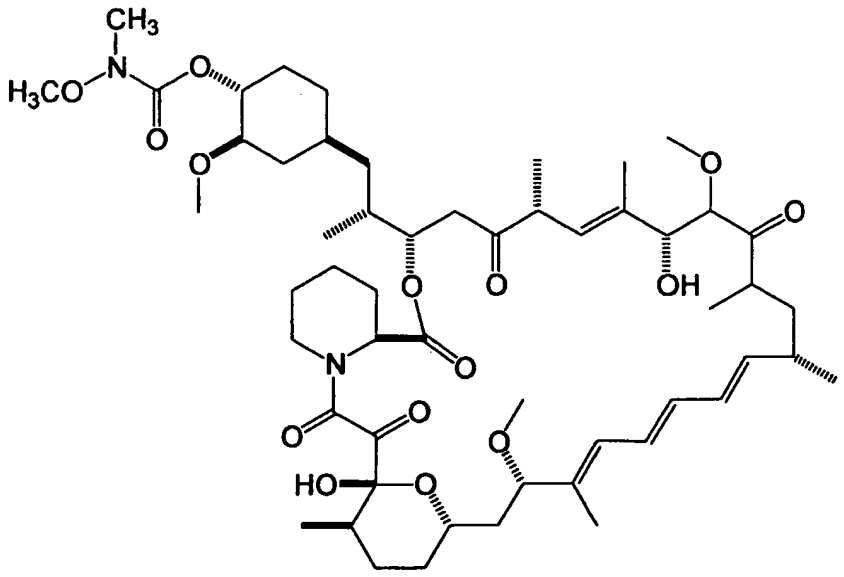
11. The medical device of claim 9, wherein said supporting structure further comprises a coating, said coating containing said therapeutic substance.

12. The medical device of claim 11, wherein said coating is polymeric.

13. The medical device of claim 12, wherein said polymeric coating is biostable.

14. The medical device of claim 12, wherein said polymeric coating is biodegradable.

15. A medical device comprising a supporting structure and the therapeutic substance



or a pharmaceutically acceptable salt or prodrug thereof.

16. The medical device of claim 15, wherein said supporting structure is selected from the group consisting of coronary stents, peripheral stents, catheters, arterio-venous grafts, by-pass grafts, and drug delivery balloons used in the vasculature.

17. The medical device of claim 15, wherein said supporting structure further comprises a coating, said coating containing said therapeutic substance.

18. The medical device of claim 17, wherein said coating is polymeric.

19. The medical device of claim 18, wherein said polymeric coating is biostable.

20. The medical device of claim 18, wherein said polymeric coating is biodegradable.

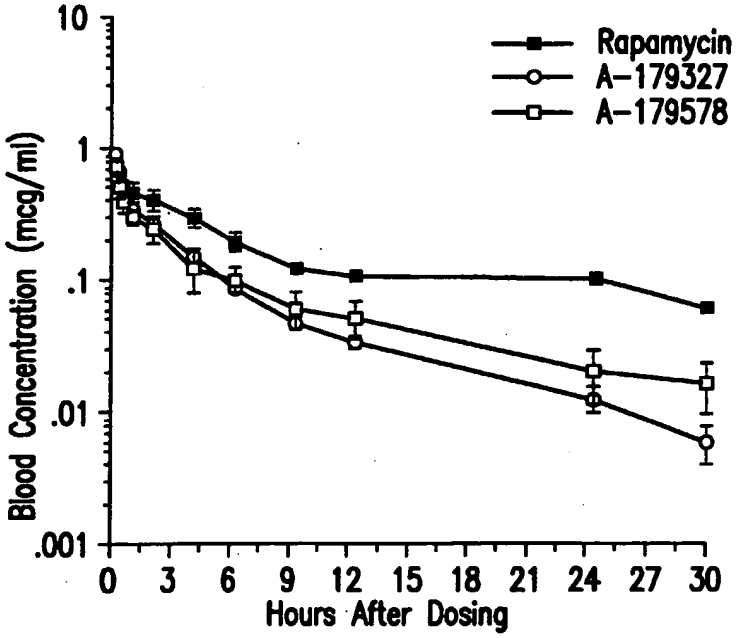

10
1
.1
.01
.001
Blood Concentration (mcg/ml)
0 3 6 9 12 15 18 21 24 27 30
Hours After Dosing
Rapamycin
A-179327
A-179578

FIG.1

The Biodiv*Ysio* PC-coated Stent

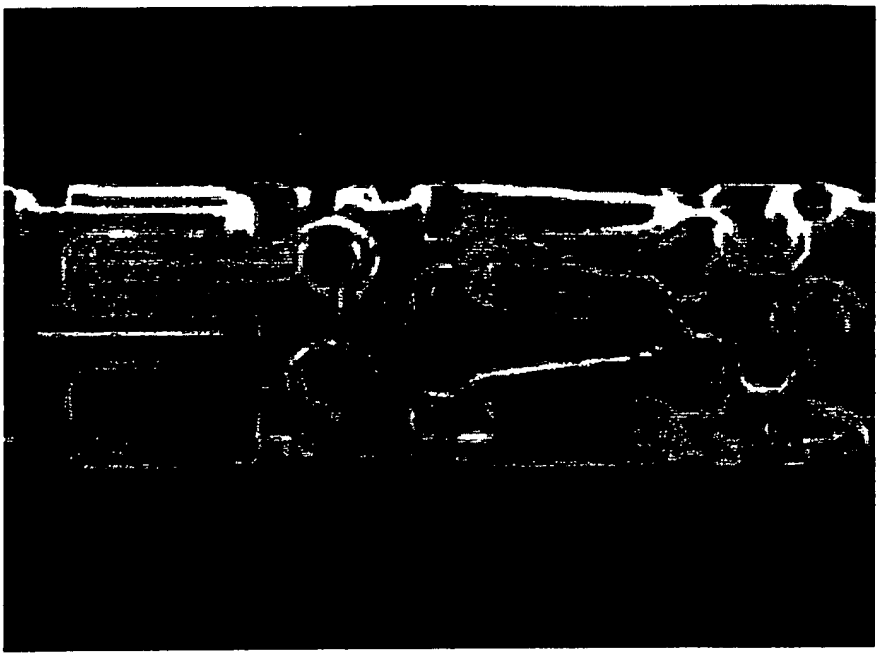


FIG.2

Polymer Only

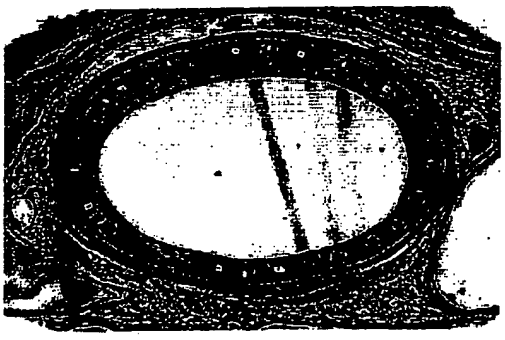


FIG.3A

Polymer plus Drug

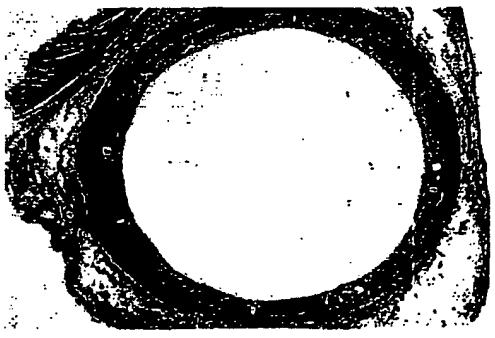


FIG.3B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 02/28798

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L31/16 A61L27/54

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L A61K A61F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 273 913 B1 (KING KRISTEN ET AL) 14 August 2001 (2001-08-14) column 4, line 64-66 column 5, line 21-24 column 5, line 65 -column 6, line 45	1-20
A	US 6 015 815 A (MOLLISON KARL W) 18 January 2000 (2000-01-18) abstract; examples column 8, line 51-66 column 10, line 47-64 column 11, line 5-12,47-62 column 12, line 13-41	1-20
X,P	EP 1 236 478 A (MEDTRONIC AVE INC) 4 September 2002 (2002-09-04) claim 25	1-20

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 December 2002	30/12/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Böhm, I

4

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 02/28798

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 01 87372 A (CORDIS CORP) 22 November 2001 (2001-11-22) page 5, line 9-26 page 7, line 13-30 claims	1-8
A,P	WO 02 066092 A (BOURGUIGNON BERNARD ;LAWRENCE MARCUS F (CA); ANGIOGENE INC (CA); L) 29 August 2002 (2002-08-29) page 3, line 15 -page 8, line 3 claims 8,29	1-8

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 02/28798

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6273913 B1	14-08-2001	EP 0950386 A2	20-10-1999
		US 2001029351 A1	11-10-2001
		US 2001027340 A1	04-10-2001
US 6015815 A	18-01-2000	US 6329386 B1	11-12-2001
		US 2002123505 A1	05-09-2002
EP 1236478 A	04-09-2002	EP 1236478 A1	04-09-2002
		US 2002127263 A1	12-09-2002
WO 0187372 A	22-11-2001	AU 5977401 A	26-11-2001
		AU 6157901 A	26-11-2001
		AU 6158001 A	26-11-2001
		AU 6158101 A	26-11-2001
		AU 6295701 A	26-11-2001
		AU 6311201 A	26-11-2001
		AU 6311301 A	26-11-2001
		WO 0187372 A1	22-11-2001
		WO 0187373 A1	22-11-2001
		WO 0187374 A1	22-11-2001
		WO 0187342 A2	22-11-2001
		WO 0187375 A1	22-11-2001
		WO 0187263 A2	22-11-2001
		WO 0187376 A1	22-11-2001
		US 2001029351 A1	11-10-2001
		US 2002016625 A1	07-02-2002
		US 2002007213 A1	17-01-2002
		US 2002007214 A1	17-01-2002
		US 2002007215 A1	17-01-2002
		US 2002005206 A1	17-01-2002
WO 02066092 A	29-08-2002	WO 02066092 A2	29-08-2002
		US 2002119178 A1	29-08-2002